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A testing time for koalas

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Abstract: Addressing the threat posed by chlamydial infection to koalas in Australia: Use of rapid diagnostic tests in the field.

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1 **Guest Editorial**

2 **Addressing the threat posed by chlamydial infection to koalas in Australia: Use of rapid** 3 **diagnostic tests ‘in the field’**

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5 The paper by Hanger et al. (2012) published in this issue of *TVJ* compares the
6 performance of a rapid antigen detection test with that of a quantitative PCR assay to detect
7 chlamydial infections in koalas (*Phascolarctos cinereus*). Chlamydial infections are
8 extremely important in wild koalas, as they have the potential to drive this already-threatened
9 animal to extinction. Chlamydiosis has contributed, in combination with habitat loss, climate
10 change and injuries caused by cars or dogs, to both the rapid decline and localised extinction
11 of the wild koala population. In fact, chlamydial keratoconjunctivitis was suggested to be the
12 possible cause of the decline in the koala population between 1885 and 1930 in Eastern
13 Australia (Cockram and Jackson, 1981). Alarming, in Queensland, surveys from 2001 to
14 2008 suggest that koala numbers have dropped as much as 45% and 15% in urban areas and
15 ‘bushland’, respectively.

16
17 Koalas can become infected with two different chlamydial species, *Chlamydia pecorum*
18 and *C. pneumoniae*. The koala biovar of *C. pneumoniae* causes rhinitis and pneumonia,
19 whereas infection with *C. pecorum* leads to more severe diseases like keratoconjunctivitis
20 possibly leading to blindness. In female koalas, *C. pecorum* can cause vaginitis, cystitis and
21 ovarian cysts with infertility as a possible sequela. The separation of the family
22 *Chlamydiaceae* into two genera, *Chlamydia* and *Chlamydophila*, based on 16S rRNA
23 sequence analysis was proposed by Everett et al. (1999). As this taxonomic division has not
24 subsequently been consistently used in the literature, and has generally not been accepted by

the scientific community, it was recently proposed to abandon the genus name *Chlamydophila* and to transfer all the current *Chlamydophila* spp. into one genus *Chlamydia*.

In the current nomenclature (Kuo et al., 2011), the family *Chlamydiaceae* contains nine species: *C. trachomatis*, *C. pneumoniae*, *C. psittaci*, *C. pecorum*, *C. abortus*, *C. suis*, *C. felis*, *C. caviae*, and *C. muridarum*. *C. pecorum* has been isolated from ruminants, swine and koala (Pospischil et al., 2010). Strains detected in koalas are monophyletic in nature, as demonstrated when using novel molecular markers (Marsh et al., 2011). The species *C. pneumoniae* includes three biovars: human, horse and koala (Kuo et al., 2011). Whereas human isolates are essentially clonal, isolates of animal origin (amphibian, reptilian, equine and marsupial) are much more diverse and at least two separate animal-to-human cross-species transfer events are suggested to have occurred in the evolution of this pathogen (Mitchell et al., 2010).

The outcome of field studies depends greatly on the availability and proper collection of field samples. Swabbing of different anatomical sites is best performed using ‘flocked’ swabs (Chernesky et al., 2006) or cytobrushes (Polkinghorne et al., 2009), to obtain the epithelial cells that harbour these obligate intracellular organisms. The type of sample submitted, the amount of DNA/viable organisms present in the sample, the preservation and possible contamination of the sample, and the length and type of transportation are crucial factors influencing the correct diagnosis of chlamydial infections. Importantly, recently developed rapid tests enable direct field-testing and diagnosis of chlamydial infections without transportation of samples. Many commercially available rapid antigen detection tests have been developed over the last 25 years primarily for the detection of *C. trachomatis*

infection in humans. As most of these tests are based on the family-specific LPS antigen they are also suitable for the detection of other chlamydial species.

In a comparison of nine antigen detection kits for the detection of chlamydial urogenital infections in koalas, the Clearview test performed best with a sensitivity of 91% (Wood and Timms, 1992). Contrasting results were obtained with the same test when it was used to detect *C. abortus* in ovine fetal membranes (Wilsmore and Davidson, 1991), and *C. psittaci* in conjunctival and cloacal samples from turkeys (Vanrompay et al., 1994), respectively. These inconsistencies can probably be explained by variation between the sampling sites and correlation with infectious load, as reported by Hanger et al. (2012) in this issue of *TVJ*. In the laboratory, nucleic acid amplification tests (NAATs) remain the ‘gold standard’ for detecting chlamydial infections in domestic and wild animals (Sachse et al., 2009). Under field conditions, rapid tests may be helpful in obtaining a prompt diagnosis of active chlamydial infection. Unfortunately, subclinically infected animals shedding low levels of organisms may not be detected. Furthermore, differentiating *C. pneumoniae* from the more prevalent and pathogenic *C. pecorum*, or the detection of other *Chlamydia* spp. or *Chlamydia*-like organisms, or mixed chlamydial infections is not possible using such assays.

Despite these limitations, the Clearview assay may be a useful adjunct test in diagnosing active chlamydial infections in koalas due to its portability, ease-of-use and short turn-around time (Hanger et al., 2012). Rapid tests in the field might also be useful to screen animals prior to treatment or vaccination. Carey et al. (2010) tested a multi-subunit vaccine against *Chlamydia* spp. that was found to be safe and effective in healthy female koalas. Early trials of whole cell vaccines to prevent trachoma were shown to have the potential to enhance inflammation when vaccination was performed in naturally-infected individuals (Huston et

al., 2012). Thus, vaccination of subclinically infected koalas may have an adverse clinical outcome under certain circumstances, which increases the importance of rapid and accurate screening prior to vaccination. It is also important to consider that detection of subclinically infected animals can be confounded by false-negative Clearview test results. The prerequisite need to only vaccinate healthy koalas was circumvented very recently in a study by Kollipara et al. (2012), where the multi-subunit vaccine tested did not exacerbate existing lesions when administered to previously infected koalas.

In summary, the study by Hanger et al. (2012) highlights that NAATs remain the gold standard in diagnosing chlamydial infections in animals and humans, but field conditions have to be considered when using alternative ‘on-site’ rapid tests. In ‘field’ settings the handling and treatment of captive or wild koalas can be problematic, and appropriate sample collection, preservation and transportation can be challenging. Despite detailed planning, the outcome of field studies can be hampered by logistical problems and careful consideration is required before such studies are performed on wild and captive koalas or indeed on other wildlife species.

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